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Targeting the Androgen Signaling Axis in Prostate Cancer

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ABSTRACT

Activation of the androgen receptor (AR) and AR-driven transcriptional programs is central to the pathophysiology of prostate cancer. Despite successful translational efforts in targeting AR, therapeutic resistance often occurs as a result of molecular alterations in the androgen signaling axis. The efficacy of next-generation AR-directed therapies for castration-resistant prostate cancer has provided crucial clinical validation for the continued dependence on AR signaling and introduced a range of new treatment options for men with both castration-resistant and castration-sensitive disease. Despite this, however, metastatic prostate cancer largely remains an incurable disease, highlighting the need to better understand the diverse mechanisms by which tumors thwart AR-directed therapies, which may inform new therapeutic avenues. In this review, we revisit concepts in AR signaling and current understandings of AR signaling-dependent resistance mechanisms as well as the next frontier of AR targeting in prostate cancer.

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INTRODUCTION

Androgens are essential hormones in the maintenance of normal male physiology and sex differentiation, including in the prostate.[1,2] Activation of the androgen receptor (AR) is a hallmark of prostate cancer, in which AR-driven transcriptional programs can instigate and support tumor growth. Recognition of this dependence dates back to the original observations made by Charles Huggins and Clarence Hodges in the 1940s that surgical castration induced tumor regression, thus proving the pathophysiologic reliance on androgens and pioneering the therapeutic targeting of AR in this sex-biased disease.[3,4] For this seminal discovery, Huggins was awarded the Nobel Prize in Physiology or Medicine in 1966.

Although targeting AR in prostate cancer has been a translational success story, resistance inevitably arises, often driven by molecular alterations in the androgen signaling axis. With the advent of multiple effective next-generation AR-directed therapies, the landscape of resistance mechanisms has become increasingly diverse and complex. Furthermore, the reality that metastatic prostate cancer largely remains an incurable malignancy despite targeting the apparent Achilles heel of this disease indicates an unmet need to understand how tumors evade AR-directed therapies. In this review, we will revisit concepts in AR signaling, with a perspective focused on the next frontier of AR targeting in prostate cancer.

OVERVIEW OF AR ACTION

The AR is a ligand-dependent transcription factor and member of the steroid receptor family consisting of the estrogen receptor, progesterone receptor (PR), glucocorticoid receptor (GR), and mineralocorticoid receptor (MR).[5] These steroid receptors share varying degrees of homology but are functionally distinct, with unique actions dictated primarily by the specificity of cognate ligand binding and differential transcriptional programs.[6] Under physiological conditions, the principal androgenic ligands for AR are testosterone and its more potent 5α-reduced derivative, dihydrotestosterone (DHT).[7] In the absence of ligand, the inactive AR generally resides within the cellular cytoplasm bound by chaperone proteins. Androgen binding triggers conformational changes that promote AR nuclear translocation, homodimerization, binding to DNA at androgen response elements, and direct transcriptional activation of target genes (Fig 1).[6,8,9]

THERAPEUTIC TARGETING OF AR IN PROSTATE CANCER—A HISTORICAL PERSPECTIVE

Since the initial demonstration by Huggins and Hodges, depletion of gonadal testosterone by surgical or medical castration (also referred to as androgen deprivation therapy [ADT]) has remained a mainstay of therapy for prostate cancer. Evidence for AR activation in promoting prostate cancer even in early stages of disease derives from two key observations. First, prostate-specific antigen (PSA) is frequently elevated at diagnosis and often rises to herald disease progression. The *KLK3* gene encoding PSA is a direct transcriptional target of AR.[10] Second, AR-driven transcription can be hijacked by genomic rearrangements that fuse regulatory elements from AR target genes with proto-oncogene gene bodies, thereby coupling physiologic AR signaling with dysregulated oncogenic pathways.[11] The prototypical example is TMPRSS2-ETS fusions, which are among the most common and earliest genomic aberrations found in

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CONTEXT

Key Objective

What are important molecular and metabolic variants in the androgen signaling axis that can arise in the context of therapeutic resistance in prostate cancer?

Knowledge Generated

The molecular landscape of therapeutic resistance to androgen receptor (AR)–directed therapies in prostate cancer is characterized by multiple changes converging on the androgen signaling axis, which include perturbations to AR, to androgen biosynthesis, or to downstream AR-regulated oncogenic pathways. Understanding these different mechanisms may inform investigations into novel treatment approaches for prostate cancer.

Relevance *(M.A. Carducci)*

AR biology, signaling, and targeting has been the mainstay of prostate cancer therapy, yet new knowledge impacts how we monitor current approaches and develop new strategies in order to tailor and improve outcomes of men with advance prostate cancer. This review places the current biology and evolving treatment approaches/combinations in a practical and clinical focus.*

*Relevance section written by *JCO* Associate Editor Michael A. Carducci, MD, FACP, FASCO.

primary prostate cancer and precancerous lesions.[12,13] However, despite this seemingly vital role of AR signaling, alterations in AR are rare in primary prostate cancer and untreated metastatic disease, indicating that AR signaling is necessary but not sufficient alone to drive early tumor development.[14-19]

Although initially effective, the response to ADT is invariably followed by recurrence of castration-resistant prostate cancer (CRPC). For decades, the prevailing dogma was that CRPC represented an androgen-independent state, although it is now appreciated that inappropriate restoration of the androgen signaling axis occurs in most cases of CRPC to drive disease progression. This is supported by the fact that AR is frequently overexpressed in CRPC, with a rise in PSA usually accompanying its onset. In early investigations, it became clear that AR overexpression could promote tumor proliferation in response to castrate levels of androgens,[20-22] the clinical importance of which is underscored by the high frequency of *AR* gene amplification in tumors after hormonal therapy.[16-18] Importantly, despite castrate levels of serum testosterone, tissue depletion of androgens is incomplete after ADT.[23-26] This is likely due to intratumoral androgen production from alternative steroidal precursors such as adrenal androgens.[24,27,28] The recognition of diverse resistance mechanisms involving AR has been the impetus for designing more potent AR signaling inhibitors (Fig 2).[20,30]

A pivotal milestone in the development of AR-targeting agents was achieved with enzalutamide (formerly MDV3100), a second-generation competitive antagonist that binds to AR with 5-8 fold greater affinity than bicalutamide.[30] In parallel, abiraterone was also developed as an irreversible inhibitor of CYP17A1—the enzyme that converts pregnenolone to dehydroepiandrosterone (DHEA), a precursor for potent androgen biosynthesis (Fig 3).[33] Both agents changed the treatment landscape for prostate cancer, demonstrating for the first time an overall survival benefit with retargeting AR in metastatic CRPC after progression on chemotherapy.[34,35] This provided critical clinical validation that CRPC continues to rely on AR, which then spurred the development of other second-generation AR antagonists, such as apalutamide and darolutamide, as well as the intensification of AR blockade in earlier disease stages, including nonmetastatic CRPC.[36-39] Additionally, the possibility of incomplete AR signaling suppression with ADT even before the clinical onset of CRPC provided rationale for combining ADT with second-generation AR-directed therapies.[40-45] Multiple phase III trials have now confirmed a clear survival benefit to these approaches, which have become standard of care. Yet, despite these translational successes, a minority of patients experience primary resistance, and most men will unfortunately experience disease progression after treatment with these agents. Nevertheless, one lesson learned from prior successes is that the pathophysiology of prostate cancer remains deeply tied to the molecular regulation of AR.

MOLECULAR MECHANISMS OF RESISTANCE TO AR TARGETING IN PROSTATE CANCER

Most commonly, CRPC overcomes AR signaling inhibition by reactivating the androgen signaling axis through various genetic and epigenetic alterations while a minority of cases can develop epigenetic alterations that bypass a requirement for AR signaling. Common alterations within the androgen signaling axis include AR overexpression and gene amplification, ligand-binding domain (LBD) mutations, structural

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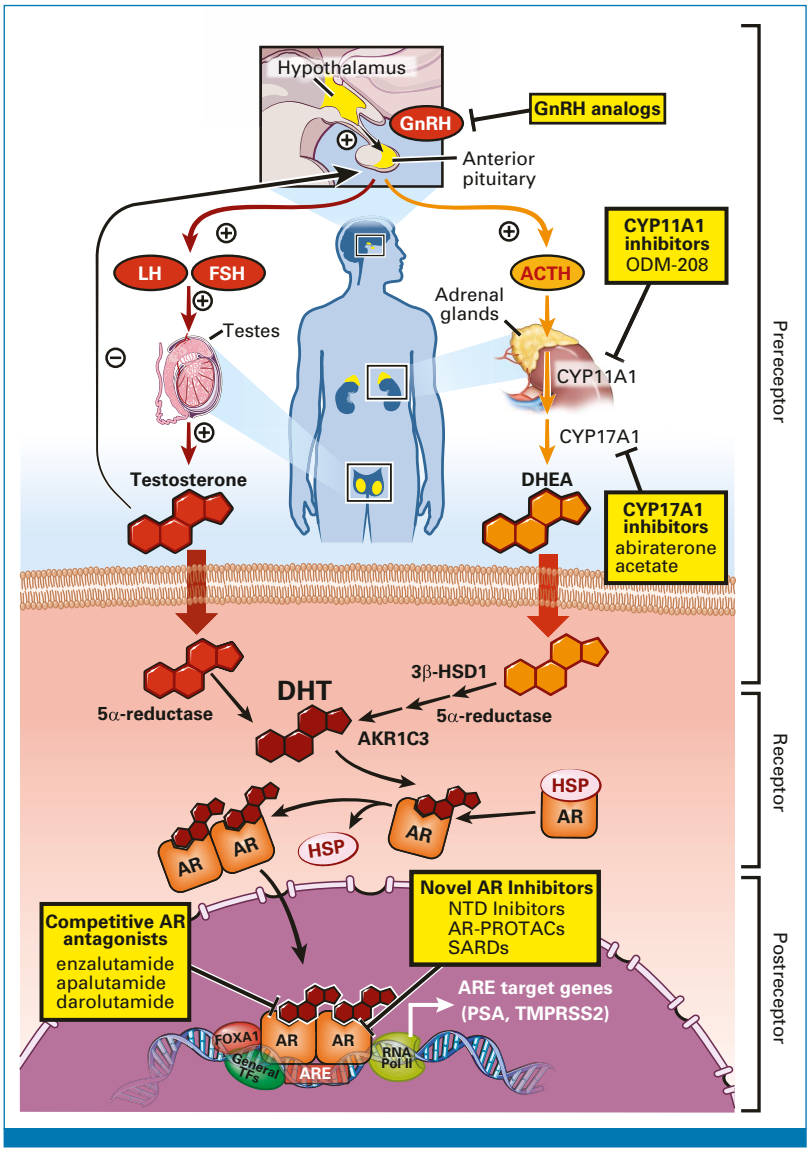


FIG 1. Androgen signaling in prostate cancer is highlighted by multiple receptor and pre/postreceptor mechanisms that serve as targets for different therapeutic approaches. Androgen biosynthesis is tightly regulated by the hypothalamic-pituitary-gonadal and hypothalamic-pituitary-adrenal axes, which govern the production of gonadal and adrenal androgens that serve as precursors for DHT, the principal AR ligand in the prostate (pre–receptor activity). On ligand binding, AR translocates from the cytoplasm to the nucleus to bind to DNA as a homodimer, permitting transactivation of target genes and pathways (postreceptor activity). Examples of different clinically approved as well as investigational inhibitors are highlighted. ACTH, adrenocorticotropic hormone; AR, androgen receptor; ARE, androgen response element; DHT, dihydrotestosterone; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; HSP, heat shock protein; LH, luteinizing hormone; NTD, N-terminal domain; PROTACs, proteolysis-targeting chimeras; PSA, prostate-specific antigen; SARDs, selective androgen receptor degraders.

rearrangements, constitutively active AR variants (AR-Vs), and alterations in pathways of androgen biosynthesis. Although alterations in AR are uncommon in primary disease, they become highly prevalent in CRPC, as is evident from multiple large-scale tissue genomic sequencing studies.[15-18,46,47] Serial sampling by plasma cell-free DNA in patients on second-generation AR-directed therapies likewise confirms that the genetic alterations arising in the setting of treatment largely converge on AR with evolving changes seen in gene copy number and structural rearrangements.[48]

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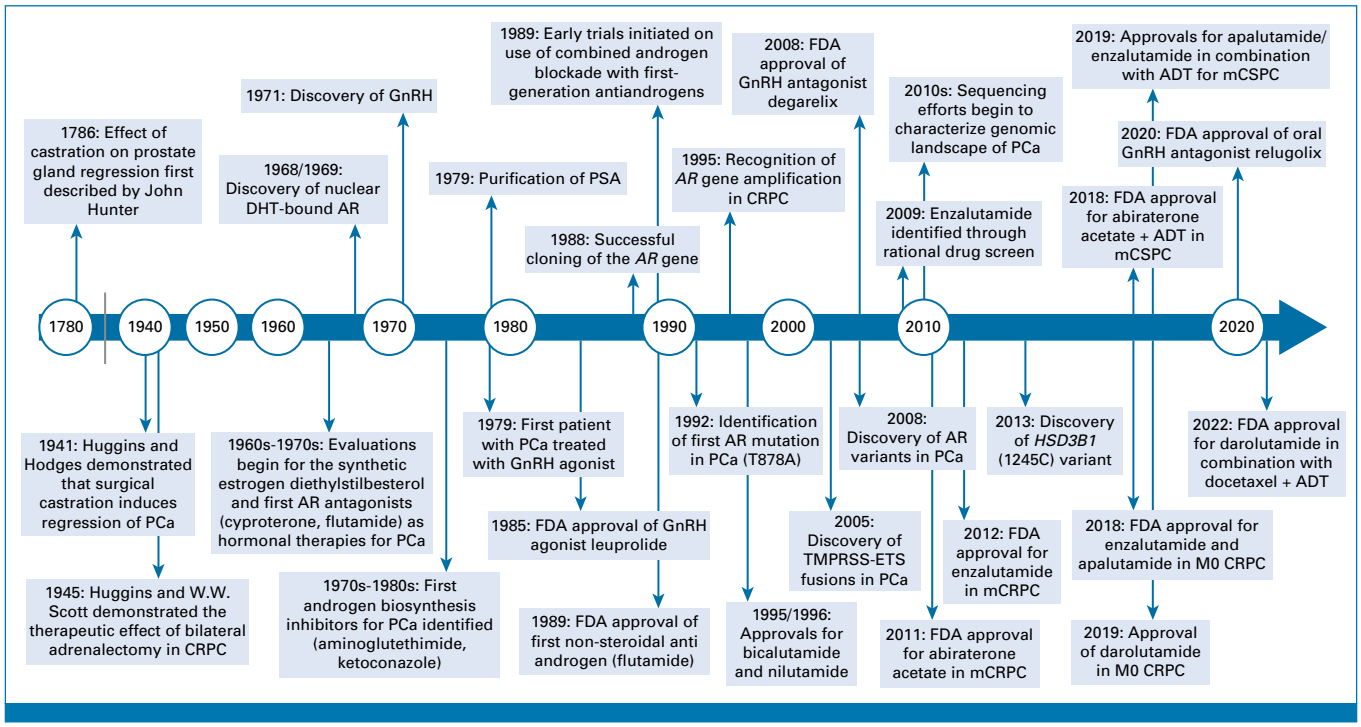


FIG 2. A timeline of key translational discoveries and therapeutic innovations in the treatment of PCa. Illustration was created with BioRender.[29] ADT, androgen deprivation therapy; AR, androgen receptor; CRPC, castration-resistant prostate cancer; DHT, dihydrotestosterone; FDA, US Food and Drug Administration; GnRH, gonadotropin-releasing hormone; mCSPC, metastatic castration-sensitive prostate cancer; PCa, prostate cancer; PSA, prostate-specific antigen.

The increasing emergence of AR-negative prostate cancers is likely an outcome of selective pressure from intensive AR suppression with second-generation AR-directed agents, forcing the reprogramming of tumor cells to survive via AR-independent pathways.[49] A subset of these tumors exhibit markers of neuroendocrine differentiation and may morphologically resemble small cell carcinoma, despite arising originally from adenocarcinoma.[49,50] A rise in the incidence of treatment-related AR-negative prostate cancers presents a unique challenge from a treatment perspective and has spurred interest in targeting AR-independent pathways or restoring AR expression in these tumors.[51] Taken together, these findings strongly suggest that AR is a master regulator of prostatic differentiation and lineage-dependent survival pathways that are subsequently usurped by prostate cancer—such that resistance to potent AR signaling blockade necessitates augmentation of AR signaling or a switch to AR-independent programs.[52]

Below, we have organized AR resistance mechanisms into two groups: (1) those which directly perturb the AR protein and (2) those that influence either the availability of steroid ligands for AR or modify AR binding/actions (later designated as prereceptor and postreceptor, respectively; Fig 1). Understanding which of these mechanisms are operative in individual tumors could inform which strategies may be most effective to overcome treatment resistance. However, one challenge is that the varied resistance mechanisms to AR-directed therapies are not necessarily mutually exclusive. Nevertheless, comprehensive characterization of these mechanisms should hopefully clarify and refine the molecular taxonomy of treatment-resistant disease.

AR STRUCTURE/FUNCTION

The *AR* gene comprises eight exons spanning 183 kb of the X chromosome at Xq11-12 and encodes a 110 kDa protein that is approximately 919 amino acids (Fig 4). Notably, *AR* gene amplification (commonly by tandem duplication) is the most frequent molecular alteration in CRPC, occurring in about approximately 60%-70% of cases.[46] Furthermore, the amplicon often encompasses both the AR gene body as well as an enhancer site approximately 650 kb centromeric to *AR*.[53,54] However, in approximately 10%-15% of cases, amplification of this enhancer can occur independently of the *AR* gene body, which drives AR overexpression similar to *AR* gene body amplification.[54] Interestingly, this region displays the epigenetic hallmarks of a developmental enhancer that is potentially reactivated in CRPC.[53] Similar to gene amplification, structural rearrangements in *AR* are also common in CRPC, occurring in approximately 13%-33% of patients before abiraterone or enzalutamide treatment and increasing in frequency to approximately 25%-50% after treatment.[55,56] These structural rearrangements can occur concomitantly with or independent of *AR* amplification and

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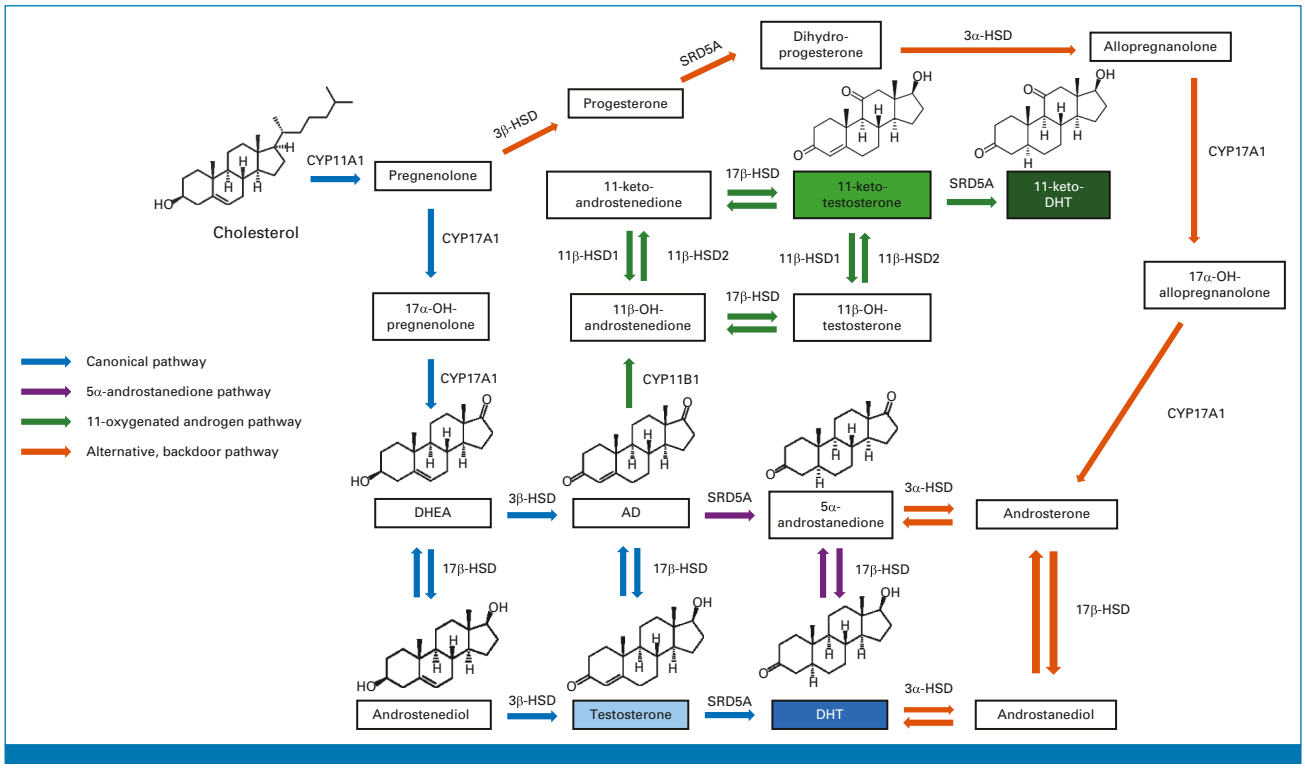


FIG 3. Several key pathways of steroidogenesis contributing to androgen biosynthesis, which include the canonical pathway of DHT synthesis through testosterone (blue) as described in normal physiology, as well as the 5α-androstanedione (5α-dione) pathway, which is the primary route of DHT biosynthesis from adrenal precursors in prostate cancer.[31,32] More recently, the 11-oxygenated androgen pathway has gained increasing recognition for generation of 11-keto-testosterone and 11-keto-DHT, which can serve as bona fide AR agonists in prostate cancer (green). In addition, multiple other potentially relevant pathways exist, including the alternative, backdoor pathway (orange). Of note, this simplified schematic is not comprehensive in depicting all possible pathways, including those through 17α-OH progesterone derivatives as intermediates. AD, androstenedione; AR, androgen receptor; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; HSD, hydroxysteroid dehydrogenase.

can give rise to diverse AR variant proteins with constitutive activity.[55,56] Both *AR* gene amplification and structural rearrangements have been implicated in driving resistance to enzalutamide and abiraterone.[48,57,58]

Like other steroid nuclear receptors, AR comprises four major structural domains: an N-terminal domain (NTD), a DNA-binding domain (DBD), a hinge region, and a C-terminally positioned LBD (Fig 4).[6] The DBD and LBD are the most highly conserved across different species and share significant homology with other steroid receptors while the NTD is unique, possibly reflecting its specificity in AR function.[9] The NTD (exon 1) harbors a strong transcriptional activation element termed AF-1, which is the primary effector of transactivation.[59-61] Loss of the LBD manifests with constitutive activity, indicating its basal repressive role on the NTD.[62,63] In vitro studies have also suggested critical interactions between the N-terminus and C-terminus in AR transactivation, although more contemporary in vivo work suggests that this property may be dispensible.[64] In recent years, various constitutively active AR-Vs have been characterized that lack the LBD and may command AR programs in a ligand-independent manner.[65-68] Importantly, the majority of current AR-directed agents either directly interact with or require a functioning LBD and thus do not act on AR-Vs. Accordingly, development of NTD inhibitors has been an attractive concept, although the intrinsic structural disorder of this domain is a crucial biophysical property for transcriptional activity that also presents an inherent challenge for the design of inhibitors.[69] EPI-506 is a bisphenol-like compound that was developed as a covalent NTD inhibitor and recently tested in a phase I study of patients with mCRPC resistant to second-generation AR-directed therapies.[70] EPI-506 achieved only minor PSA declines, a finding that later attributed to poor bioavailability.[70,71] EPI-7386 is a successor drug with greater metabolic stability and more potent activity, which is currently undergoing investigation.[72] Notably, although these agents target the NTD, they may have broader, less specific actions that also contribute to their therapeutic effect.[73]

The DBD (exons 2-3) is the most conserved region of AR, which is perhaps unsurprising given the critical interactions with DNA required for gene expression.[9] The first zinc finger

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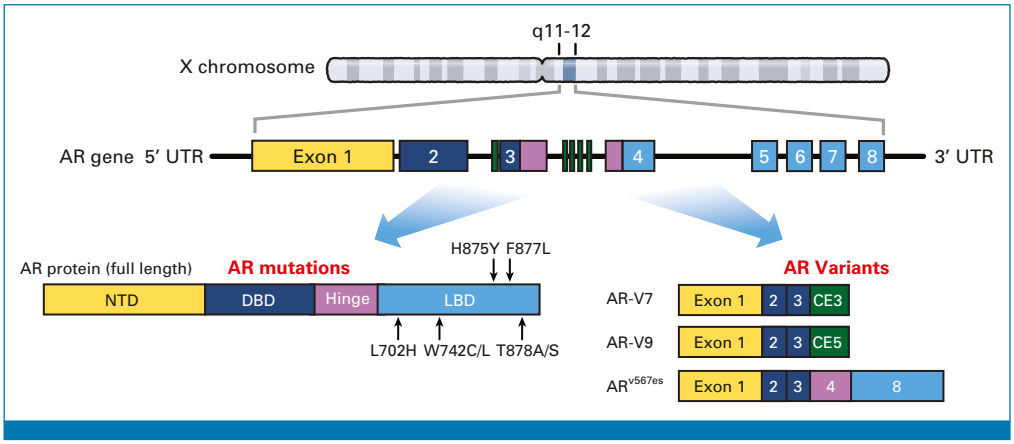


FIG 4. The AR gene locus and protein. The mRNA transcript encoding the full-length AR protein encompasses eight exons, which consists of four major functional protein domains. AR mutations most frequently occur in the LBD. Several AR-Vs including AR-V7, AR-V9, and AR-V12/ARv567es are depicted as well, including their corresponding exons. AR, androgen receptor; AR-Vs, AR variants; DBD, DNA-binding domain; LBD, ligand-binding domain; NTD, N-terminal domain; UTR, untranslated region.

of the DBD makes base-specific contacts within the major groove of DNA via a conserved series of amino acids known as the P(roximal)-box, whereas the second zinc finger mediates receptor dimerization via a similarly conserved D(istal)-box.[6] Remarkably, both the P-box and D-box residues as well as the consensus hexameric repeat sequence of DNA recognized by AR are also shared by GR, PR, and MR. This may explain some degree of overlap between the genomic binding sites of AR and GR, which is pertinent in CRPC, as GR appears to be upregulated in certain enzalutamide-resistant tumors, leading to GR binding to and transcriptionally regulating a subset of AR target genes.[74,75]

The DBD and LBD are joined by a flexible hinge region (exons 3-4). The hinge region contains target sites for post-translational modifications and a bipartite nuclear localization signal that orchestrates nuclear import.[6] The LBD (exons 4-8) governs ligand-dependent AR activity, illustrated by the fact that deletion of this region renders the AR constitutively active and unresponsive to androgens.[62] In prostate cancer, the LBD is the most frequent site of gain-of-function mutations. In addition to the ligand-binding pocket, the LBD also contains an AF-2 element which enables interaction with AR coregulators, a nuclear export signal that excludes unliganded AR from the nucleus, and an allosteric BF-3 regulatory site.[76-78] These sites are potential targets for noncompetitive inhibitors, which remain an active area of investigation.[79-81]

AR MUTATIONS

AR mutations detected in prostate cancer typically arise after exposure to antiandrogens. The majority of these are gain-of-function missense mutations concentrated within the LBD that enable receptor promiscuity and inappropriate activation by a broad range of noncanonical ligand partners or even antagonists. Contemporary next-generation sequencing methods have shown that four hotspot LBD mutations (L702H, W742C/L, H875Y, and T878A/S) encompass a significant number of cases, together being found in approximately 10%-25% of CRPC.[16-18,47,82] The first AR mutation described in prostate cancer was T878A, identified initially in the LNCaP cell line (derived from a man with CRPC) after the observation that hydroxyflutamide was an agonist in this model and later confirmed in a patient with CRPC.[83-85] In vitro functional characterization of T878A/S and H875Y revealed that these mutations confer increased AR activation in response to various noncanonical steroidal ligands such as progesterone, estradiol, and DHEA, as well as to antiandrogens such as flutamide.[86-89] Similarly, AR W742C/L can be activated by bicalutamide.[90,91] The ability of these mutations to grant modest agonist potential to antagonists is the purported mechanism of antiandrogen withdrawal syndrome, a phenomenon initially described with first-generation antiandrogens, wherein discontinuation of therapy leads to PSA declines.[90,92,93]

Several mutations, including a more recently described F877L mutation, have potential to promote resistance to second-generation AR-directed therapies.[94-97] In vitro studies suggest that F877L can confer agonist activity to enzalutamide and apalutamide (previously ARN-509), although this mutation occurs infrequently in patients overall and does not appear to be enriched by treatment.[98] Notably, in contrast to enzalutamide and apalutamide, darolutamide (previously ODM-201) bears a distinct chemical structure with inhibitory activity even in enzalutamide-resistant models that harbor AR F877L or other resistance mutations.[99] However, the optimal sequencing of treatment with AR-directed agents including darolutamide in the context of AR mutations remains to be determined.

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AR T878A, H875Y, and L702H mutations have similarly been observed in patients experiencing disease progression on abiraterone.[57,58,100] These mutations may hinder efficacy by enabling AR to be activated by noncanonical ligands such as progesterone and other steroids synthesized upstream of CYP17A1, which are thus not suppressed by abiraterone. This has prompted interest in the development of steroid biosynthesis inhibitors that target enzymatic steps upstream of CYP17A1 (discussed below). Of note, the L702H mutation, alone or in combination with T878A, appears to be activated by glucocorticoids, which is a largely unavoidable obstacle given that abiraterone requires concurrent glucocorticoid administration to prevent mineralocorticoid excess. Furthermore, given their steroidal structure, abiraterone and its metabolites can also directly bind to AR to influence AR activity, which could explain some degree of cross-resistance between AR antagonists and abiraterone.[101-104]

AR-Vs

A number of AR-Vs have been described which lack the LBD and can thus maintain AR signaling in a constitutive, ligand-independent manner. AR-Vs generally arise via alternative RNA splicing of intronic sequences or through structural rearrangements in the *AR* gene which promote altered RNA splicing patterns. To date, more than 20 AR-Vs have been identified.[65-68] Among those arising from alternative RNA splicing of intronic sequences, AR-V7 appears to be the most abundant in CRPC and is encoded by splicing of *AR* exons 1-3, followed by a cryptic exon CE3. In vitro, AR-V7 has been shown to either homodimerize or heterodimerize with full-length AR (AR-FL) to mediate gene transcription.[105-107] Expression of AR-V7 increases after ADT and correlates strikingly with inferior clinical outcomes after enzalutamide and abiraterone therapy, which has now been validated across multiple cohorts.[108-112] In light of this, AR-V7 may serve as a useful predictive biomarker, although how this dictates alternative treatment selection and timing remains an area of active investigation.[108-110] Other AR-Vs detected in prostate cancer tissues include AR-V9, which is similarly encoded by RNA splicing of *AR* exons 1-3 followed by a cryptic exon CE5, as well as AR-V12 (also referred to as AR^{v567es}), arising from structural rearrangement and skipping of exons 5, 6, and 7 (Fig 4). Like AR-V7, detection of AR-V9 in CRPC biopsies may predict for resistance to abiraterone.[113] However, given that expression of AR-V7 and AR-V9 generally mirrors that of AR-FL, ongoing and unresolved questions remain regarding whether AR-Vs drive CRPC independent of AR-FL, as well as if AR-Vs activate similar or different transcriptional programs compared with AR-FL (with data to support both conclusions).[105-107,114] The identification of certain *AR* gene structural rearrangements in CRPC tissues that block expression of AR-FL while promoting AR-Vs indicates that, in specific circumstances, AR-Vs could drive therapeutic resistance.[55,56]

Given the multiple resistance mechanisms which circumvent effective targeting of the LBD, there has been interest in alternative approaches to AR signaling inhibition. In addition to the aforementioned NTD inhibitors, proteolysis-targeting chimeras (PROTACs) and selective androgen receptor degraders (SARDs) have recently emerged as novel and promising therapeutic strategies. PROTACs are heterobifunctional molecules consisting of two ligands connected by a central linker; one ligand binds to AR while the other recruits an E3 ubiquitin ligase to facilitate ubiquitination and proteasome-mediated degradation. ARV-110 is a first-in-class PROTAC, for which phase I/II data were recently reported and appears to show encouraging clinical activity among patients with heavily pretreated mCRPC, particularly among those with detectable T878A/S and H875Y mutations.[115] Similarly, several SARDs have also shown activity in preclinical models for CRPC, including against AR-V7, and could represent a new class of AR-directed therapies.[116,117]

INTRATUMORAL ANDROGEN BIOSYNTHESIS (prereceptor mechanisms)

The biosynthesis of all steroid hormones begins with 27-carbon cholesterol, which can undergo stepwise enzymatic modification, first to downstream 21-carbon steroids (progestins), followed by further conversion to 19-carbon androgens. In men, the major circulating androgens in serum are testosterone and DHEA, predominantly produced by the testes and adrenal glands, respectively.[118] As ADT does not influence the production of extragonadal androgens, CRPC can engage in intracrine androgen biosynthesis via alternative androgenic precursors to maintain AR signaling despite castrate serum levels of testosterone. Importantly, adrenal-derived DHEA can be readily metabolized to DHT (the principal AR ligand in the prostate) via a limited repertoire of enzymes expressed within prostatic tissue (Fig 3).[119] These steroidogenic enzymes are frequently upregulated in CRPC to enable more efficient androgen biosynthesis of AR ligands.[25,28]

Multiple biosynthetic pathways converge on DHT as the final active metabolite (Fig 3).[120] Although targeting of these enzymes is appealing given their requirement for androgen production, one consideration for therapeutic development is that inhibiting enzymes more proximally in the pathway may inadvertently disrupt synthesis of other physiologically indispensable steroids (such as mineralocorticoids and glucocorticoids), while blocking more distal enzymes spares the generation of upstream metabolites and creates opportunities for escape mechanisms. For instance, despite inhibition of CYP17A1 (17α-hydroxylase/17,20-lyase) activity, abiraterone does not prevent the generation of progestins that can activate AR in the context of specific AR mutations.[100] Although there has been interest in inhibiting CYP11A1 upstream to overcome this issue, this maneuver mandates glucocorticoid and mineralocorticoid replacement

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therapy. In phase I/II trials, the first-in-class CYP11A1 inhibitor ODM-208 was more effective in patients with detectable AR LBD mutations in achieving PSA declines but was associated with grade 3 adrenal insufficiency at higher doses.[121,122] Thus, striking a balance between blockade of AR mutants while allowing for physiologic glucocorticoid/mineralocorticoid signaling appears to be a challenge with CYP11A1 inhibition.

Immediately downstream to CYP17A1 is 3β-hydroxysteroid dehydrogenase (3β-HSD), which catalyzes the rate-limiting step in the conversion of DHEA to androstenedione (AD). A germline variant in 3β-HSD1 (encoded by *HSD3B1*, the predominant isoenzyme expressed in the prostate) renders this enzyme resistant to ubiquitin-mediated degradation and increases protein stability, with resultant increased metabolic flux of DHEA to downstream androgens.[123] Inheritance of *HSD3B1* (1245C), the adrenal-permissive allele that encodes for the more stable form of 3β-HSD1, has been associated with rapid onset of resistance to ADT and poorer clinical outcomes in CRPC, which has been independently validated across several different cohorts.[124-128] In addition, CRPC tumors from patients who are germline heterozygotes can acquire a second somatic mutation or undergo loss of heterozygosity.[123] Recent evidence also indicates that 3β-HSD1 activity may require phosphorylation by the tyrosine kinase BMX, a finding that could present novel therapeutic avenues to modulate androgen biosynthesis.[129]

Conversion of AD to DHT requires two final reactions mediated by 17β-hydroxysteroid dehydrogenase and 5α-reductase family enzymes. In prostate cancer, AKR1C3 (type 5 17β-hydroxysteroid dehydrogenase) is overexpressed in response to ADT.[28,130,131] However, development of potent, selective AKR1C3 inhibitors is challenging given the sequence similarity of AKR1C3 to several other enzymes within this family, of which inhibition could lead to potentially undesirable effects.[132] Furthermore, although 5α-reductase inhibitors are routinely used in the treatment of benign prostatic conditions, their role in prostate cancer is less clear, especially as blockade can result in unintended upstream accumulation of testosterone to potentially rescue AR activity.[133,134]

Beyond these well-described pathways, other alternative, underappreciated biosynthetic pathways likely also exist that are relevant in prostate cancer (Fig 3).[120] For instance, C19 steroid 11β-OH derivatives of AD can be metabolized by CRPC into 11-keto-testosterone/11-keto-dihydrotestosterone, which can act as bona fide AR agonists (Fig 3).[135,136] Aberrant cortisol metabolism via dysregulation of 11β-HSD2 may also promote upregulation of GR signaling to bypass AR and mediate enzalutamide resistance.[137,138] Ultimately, effective therapeutic blockade of androgen biosynthesis requires understanding these different pathways and their contributions toward restoring or circumventing AR activity.

POSTRECEPTOR MECHANISMS

In light of multiple prereceptor and receptor-level resistance mechanisms that promote continued AR activity, a potentially favorable approach might be to target postreceptor mechanisms, which include AR binding and activation of specific downstream genes or oncogenic pathways modulated by AR. This requires a deep understanding of specific transcriptional programs directed by AR, as well as how AR-dependent transcription is regulated by a variety of coregulators.[139] For example, the AR cistrome undergoes extensive reprogramming with malignant transformation and progression.[140-142] The importance of molecular partners in this process is perhaps best exemplified by the high frequency of driver mutations in key proteins such as FOXA1 and SPOP, which have been shown to interface with AR signaling to promote prostate cancer.[17,143,144] Similarly, structural variants such as TMPRSS2-ETS fusions can hijack AR-driven programs, which may be particularly relevant in early-onset disease.[145] Some evidence also suggests that AR signaling itself can conversely provoke these nonrandom translocation events to promote carcinogenesis.[146-148] Of note, although a majority of primary prostate cancers express AR and can be characterized by a taxonomy-defining alteration, approximately 30% lack a clear driver alteration, despite clinically resembling tumors with identifiable driver alterations. Indeed, a lack of well-defined molecular correlates for Gleason grade exists, and further understanding is thus required in terms of the processes that drive aggressive primary disease.[14,149-151]

In the context of various potential cellular functions of AR, there remains interest in how to exploit these functions therapeutically. For instance, it is well-recognized that AR can engage in cross-talk with oncogenic signaling pathways such as PI3K/AKT to facilitate tumor progression[152,153] The relationship between AR and mediating DNA damage repair as well as the immune response has also prompted efforts to combine AR-directed therapies with other agents, such as PARP inhibitors or immunotherapy.[148,154-157] AR target gene expression is also strongly affected by epigenetic processes, including histone acetylation and methylation, which can modify chromatin accessibility and AR binding.[140-142] Bromodomain and extraterminal family proteins are epigenetic readers of acetylated histones that are targets for inhibitor design, given that they influence expression of prostate cancer oncogenes, including c-MYC.[158-160] Epigenetic regulation can also contribute to enzalutamide resistance because of GR upregulation or other mechanisms that regulate endogenous repeat elements.[161,162] More recently, it has become apparent that mechanisms operating in CRPC cells to restore AR activity may also manifest with divergent actions that might be therapeutically exploited.[148,156,163-165] These mechanisms are perhaps the basis for the phenomenon of bipolar androgen therapy, in which high-dose testosterone can

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paradoxically induce clinical responses in a subset of patients.[166] Although this is not an exhaustive review of the breadth of postreceptor mechanisms, it highlights a fundamental need to better understand the varied cellular functions of AR and how AR specifically orchestrates prostate cancer programs, which may yield new insights and directions in the treatment of prostate cancer.

CONCLUDING REMARKS

In conclusion, the field of prostate cancer has seen remarkable advances in the past several decades, driven in large part by our understandings of the androgen signaling axis. With this also comes a greater appreciation for the complex mechanisms employed by prostate cancers to thwart effective inhibition of AR signaling. Despite considerable progress in the development of effective next-generation AR-directed therapies, most patients will eventually develop resistance. However, recent and ongoing molecular investigations have led to unprecedented insights into AR structure and function, which has the potential to enhance therapeutic precision and galvanize newfound directions in the treatment of men with prostate cancer.

AFFILIATIONS

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Targeting the Androgen Signaling Axis in Prostate Cancer

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